Kidney International, Vol. 31 (1987), pp. 1072–1079

The role of thromboxane in cuprophan–induced pulmonary hypertension

ALFRED K. CHEUNG, ROBERT L. BARANOWSKI, and ANDREA L. WAYMAN

The Veterans Administration Medical Center, and the Department of Medicine, Medical Service University of Utah School of Medicine, Salt Lake City, Utah, USA

The role of thromboxane in cuprophan–induced pulmonary hypertension. Previous studies demonstrated that acute infusion of cuprophan activated plasma into experimental animals produce cardiopulmonary changes which included severe pulmonary hypertension. It was further suggested that these changes were mediated by complement activation products. The current study examined the role of arachidonic acid metabolites in the pathogenesis of cuprophan–induced pulmonary hypertension in the swine. Plasma thromboxane concentrations and pulmonary arterial pressure rose concomitantly with cuprophan–activated plasma infusion; both could be inhibited by the specific thromboxane synthetase inhibitor, OKY1581. Likewise, this inhibitor also blocked the increment in plasma thromboxane concentrations and pulmonary arterial pressure induced by zymosan–activated plasma. In vitro incubation of cuprophan–activated plasma with porcine lung fragments produced significantly higher thromboxane concentrations in the medium than incubation with other porcine tissues examined. It is postulated that the complement activation products formed in the plasma during cuprophan exposure subsequently stimulated release of thromboxane from the lungs and other tissues upon infusion of the plasma into animals. The thromboxane, in turn, triggers the pulmonary hypertension.

Hemodialysis with cellulosic membrane is often associated with adverse reactions such as dyspnea, chest pain, systemic hypotension or hypertension [1–3] and pulmonary hypertension [4, 5]. Experimental models utilizing swine or sheep have been developed to study the biological effects of these membrane materials as they may contribute to some of these reactions [6–8]. It had been demonstrated that acute bolus infusion of autologous plasma previously exposed to cuprophan membrane into animals produced significant leukopenia, hypoxemia, changes in systemic arterial pressure, decrease in cardiac output and severe pulmonary hypertension [6–8]. Craddock et al originally suggested that this pulmonary hypertension was the direct consequence of leukoagglutination in the pulmonary vasculature [7]. A subsequent study by Walker et al questioned whether leukocytes were necessary at all to produce this alteration in pulmonary hemodynamics [9]. Their study showed that cuprophan–activated blood could still increase pulmonary arterial pressure in leukopenic animals, and that this increase could be blocked by indomethacin.

Received for publication July 18, 1986
and in revised form October 21, 1986

Our recent study also suggested that the leukopenia and most of the cardiopulmonary changes could be reproduced by infusion of zymosan–activated plasma or purified porcine $C5a_{desArg}$ [6]. These data suggested that the anaphylatoxins generated from complement activation upon cuprophan membrane exposure were mediators of these alterations. The present studies were performed to: (a) further define the role of arachidonic acid metabolites in the pathogenesis of the cuprophan–induced pulmonary hypertension; (b) define the interactions between the arachidonic acid metabolite thromboxane and the complement activation products; and (c) identify the potential sources of this thromboxane produced in response to cuprophan–activated plasma challenge.

Methods

Animals

Normal three–breed cross swine of either sex were employed. They were of two different sizes. Larger ones weighing 25 to 35 kg were used for experiments with cuprophan–activated plasma. Smaller ones weighing 10 to 15 kg were used for experiments with zymosan–activated plasma and in vitro incubation studies. These animals were sedated with intramuscular ketamine hydrochloride and anesthetized with intravenous sodium pentobarbital before blood drawing or acute hemodynamics experiment as previously described [6]. Blood was obtained from the animals by arteriotomy and collected in two units of sodium heparin/ml whole blood. The animals were then replaced with an equal volume of normal saline and were allowed to recover from anesthesia prior to the acute experiments on the following day.

Platelet rich plasma (PRP) and platelet poor plasma (PPP)

PRP was prepared by centrifugation of heparinized blood at $200 \times g$ for 10 min. PPP was prepared by centrifugation of heparinized blood at $2{,}000 \times g$ for 20 minutes. Platelet counts in the supernatant were determined. The counts in the PPP were less than $15 \times 10^3/\mu l$. The counts in the PRP were adjusted to a standardized concentration of $250 \times 10^3/\mu l$.

Cuprophan–activated plasma (CAP)

The preparation of CAP was performed as previously described [6]. The blood compartment of a cuprophan hollow fiber dialyzer (CF-1511, Travenol Laboratory, Inc., Deerfield, Illinois, USA) was rinsed with one liter of normal saline. The dialysate compartment was filled with normal saline and clamped off. One hundred and fifty ml of platelet poor plasma was then recirculated through the dialyzer at 100 ml/min for 45 minutes at 37°C.

Zymosan–activated plasma (ZAP)

Zymosan (Sigma, St. Louis, Missouri, USA) was gently boiled in normal saline and incubated with platelet poor plasma (PPP) at a concentration of 2.5 mg/ml of PPP at 37°C for 30 minutes in the presence of 1 mM Mg^{++}. The zymosan particles were sedimented by centrifugation.

Control plasma

Control plasma was prepared as previously described [6]. Heparinized autologous plasma was incubated at 37°C for 30 minutes without other additives or stimulation.

Isolation of peripheral leukocytes

Porcine polymorphonuclear cells (PMN) and mononuclear cells (MNC) were isolated from peripheral blood using Ficoll-paque (Pharmacia Inc., Piscataway, New Jersey, USA). For MNC isolation, whole blood was diluted 1:1 with phosphate buffered saline (PBS). Ten ml of the diluted blood was layered on top of 4 ml of Ficoll-paque, and spun at 450 × g for 40 minutes at room temperature. The MNC layer at the interphase between the plasma and the Ficoll-paque was removed. The cells were then washed and spun at 200 × g for seven minutes three times to remove platelet contamination and resuspended in Tyrodes buffer.

For PMN isolation, the erythrocytes and leukocytes were allowed to settle by gravity. The buffy coat was aspirated and sedimented by centrifugation at 200 × g for seven minutes at room temperature. The contaminating erythrocytes were removed by repeated lysis with 0.2% NaCl. The leukocytes were resuspended in Tyrodes buffer, layered over an equal volume of Ficoll-paque and spun at 400 × g for 35 minutes at room temperature. The PMN layer below the Ficoll-paque was aspirated and washed.

Viability of the cells was assessed by trypan blue exclusion and purity assessed by differential counts on Wright stain of the smear. Both the viability and purity of the cell populations were >90%. The approximate yield of the MNC and PMN preparation were $2.0X10^6$/ml and $1.6X10^6$/ml of whole blood, respectively.

Preparation of other tissues

The lungs, heart, pulmonary arteries, ascending aorta, inferior vena cava, kidneys, liver and spleen were removed from the swine and placed immediately into Tyrodes buffer bubbled with 95% oxygen and 5% carbon dioxide at 4°C. Pieces of 200 mg of each of these tissues were excised and used for in vitro incubation as described below.

Acute animal experimental set–up

For acute hemodynamic studies, the animal was sedated with intramuscular ketamine hydrochloride and intravenous sodium pentobarbital. It was then intubated via a tracheostomy and placed on a volume respirator (RV-4, Ensco, Salt Lake City, Utah, USA). Intravenous normal saline was continuously given to replace the fluid loss throughout the acute study.

A balloon–wedge pressure catheter (Critikon, Tampa, Florida, USA) was placed in the pulmonary artery via the right internal jugular vein. A 4 French and a 6 French catheter was used for small and large animals, respectively. The femoral arteries and veins were cannulated with polyethylene catheters for pressure monitoring and blood sampling. Blood pressure from the femoral and pulmonary arteries were continuously recorded on a 6 channel polygraph (R612, Beckman, Fullerton, California, USA). Blood samples were collected from the femoral artery at various time intervals for leukocyte counts and determination of arachidonic acid metabolite concentrations.

Protocol for acute animal studies

After stable baseline hemodynamic readings were obtained, 0.15 ml/kg body wt of control plasma was infused intravenously as a bolus. Fifteen minutes later 0.15 ml/kg body wt of CAP was infused intravenously as a bolus. Systemic arterial blood samples were obtained at baseline, 0.5 and 3.0 minutes after the infusion. Ten minutes after the CAP injection the selective thromboxane synthetase inhibitor OKY1581 was then infused into a peripheral vein continuously at 0.05 mg/kg body wt/min for 30 minutes. The animal was then rechallenged with 0.15 ml/kg body wt of CAP. To test the dose dependent nature of the responses to OKY1581, 0.6 ml/kg body wt of CAP was then given after the pulmonary artery pressure–baseline had been reestablished. OKY1581 infusion was continued during the injection of this larger dose of CAP.

In a separate set of animals, normal saline in the same volume instead of OKY1581 was given as control. Again, 0.15 ml/kg body wt of CAP was given to the animal intravenously. Ten minutes later, saline was infused over the next 30 minutes. The same dose of CAP was then repeated.

A separate set of animals was challenged with 0.2 ml/kg body wt of ZAP intravenously. This challenge was repeated after 0.05 mg/kg body wt/min of OKY 1581 intravenous infusion for 30 minutes.

Protocol for in vitro studies

To study the potential sources of the thromboxane released in response to infusion of CAP, different porcine tissues were incubated with CAP in vitro. Fragments of different solid tissues weighing 200 mg each as described above were incubated with 5 ml of CAP or PPP for 30 minutes in a shaking water bath. The incubation was maintained at 37°C under 95% oxygen and 5% carbon dioxide. A sample of the plasma was removed before and after incubation for thromboxane determination. Lung tissues were also incubated with 1×10^{-3}M OKY1581 in additional experiments.

Five ml of CAP were also incubated with peripheral PMNs, MNCs or platelets. The final concentrations of PMNs, MNCs and platelets in the incubation were 4.6×10^3 cells/μl, 4.7×10^3 cells/μl and 250×10^3 cells/μl, respectively. For positive controls, PMNs and MNCs were incubated with a mixture of arachidonic acid (15 μM final concentration, Sigma) and calcium ionophore A23187 (6.1 μM final concentration, Sigma). Platelets were incubated with type I bovine collagen (50 μg/ml final concentration, Sigma).

Sample handling

Blood samples for leukocyte counts were placed in liquid disodium ethylenediaminetetraacetate (EDTA, 20 mM final concentration). Samples of blood or incubation media for prostaglandin E_2 (PGE_2) and thromboxane B_2(TXB_2) concentration determinations were placed immediately in indomethacin (0.1 mg/ml final concentration, Sigma) at 4°C. The plasma was separated as soon as possible and frozen at −70°C until assay.

Assays

Blood leukocyte counts as well as isolated leukocyte and platelet counts were performed on a Coulter counter (S-PLUS, Coulter Electronics, Irvine, California, USA).

Prostaglandin E_2 and TXB_2 were extracted from the plasma as follows. Five hundred μl of plasma was acidified with 1 N hydrochloric acid and applied onto a SEP-PAK C_{18} cartridge (Waters Associated, Milford, Massachusetts, USA). Petroleum ether was passed through the cartridge. The arachidonic acid cyclooxygenase products were then eluted with ethyl acetate. The concentrations of PGE_2 and TXB_2 in the eluate were determined using commercially–available radioimmunoassay kits (Seragen, Boston, Massachusetts, USA).

Statistical analysis

Values were reported as mean ±SEM. Differences in values of variables were evaluated by Student's paired or unpaired *t*-test as appropriate. *P* values <0.05 were considered as statistically significant.

Results

Animal studies with CAP

Infusion of control plasma did not produce any change in pulmonary artery pressure as reported in our previous study [6]. In contrast, acute bolus intravenous infusion of 0.15 ml/kg body wt of CAP increased the pulmonary artery pressure from 24 ± 4 to 57 ± 13 mm Hg ($P < 0.01$) within 30 seconds (Fig. 1). Concomitantly, arterial plasma thromboxane concentration increased from 0.58 ± 0.09 to 1.69 ± 0.38 ng/ml ($P < 0.05$). Thereafter, both the pulmonary artery pressure and the thromboxane concentration decreased gradually. Peripheral leukocyte counts, however, did not change significantly. The pulmonary artery pressure returned to baseline well within 10 minutes. Continuous OKY1581 infusion at 0.05 mg/kg body wt/min during the next 30 minutes did not change the pulmonary artery pressure. After the infusion of OKY1581, however, the same dose of CAP failed to increase either the pulmonary artery pressure or the plasma thromboxane concentration significantly. Again, the leukocyte counts remained unchanged. Baseline plasma PGE_2 concentration was 1.38 ± 0.17 ng/ml and remained unchanged after CAP infusion with (1.41 ± 0.10 ng/ml, $P > 0.05$) or without (1.41 ± 0.13 ng/ml, $P > 0.05$) pretreatment with OKY1581.

Figure 2 depicts the mean ±SEM of the pulmonary artery pressure of seven animals at baseline (Fig. 2A) and 0.5 minutes after CAP challenge with or without OKY1581 pretreatment (Fig. 2B,C,D). The pulmonary hypertension induced by 0.15 ml/kg of CAP (Fig. 2B) was abolished by the continuous infusion of 0.05 mg/kg/min of OKY1581 (Fig. 2C) as described

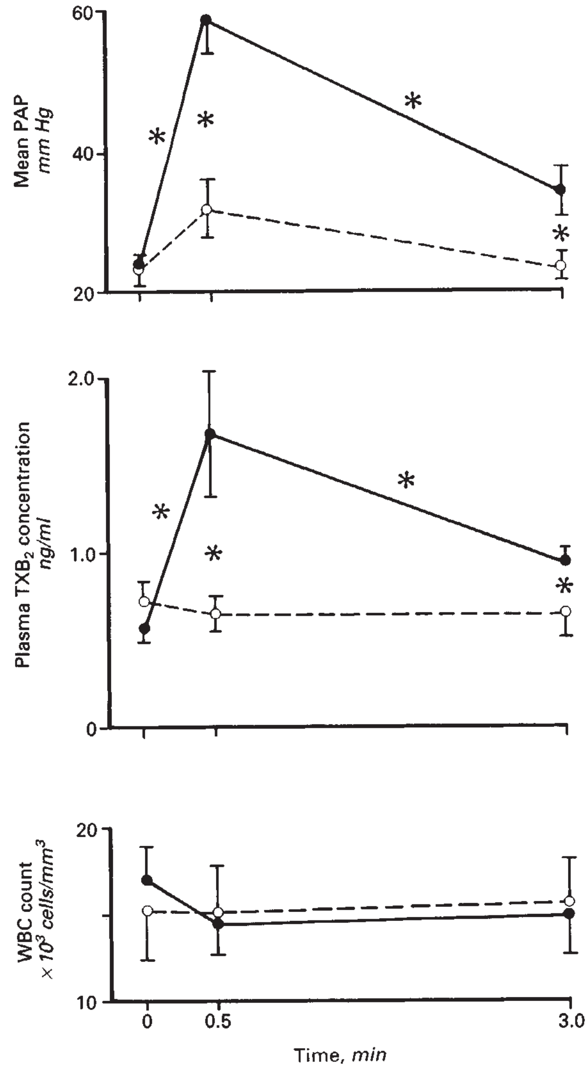

Fig. 1. *Changes in mean pulmonary artery pressure (PAP), systemic arterial plasma thromboxane B_2(TXB_2) concentration and systemic arterial leukocyte (WBC) counts in swine (N = 7) in response to acute intravenous bolus infusion of 0.15 ml/kg body wt of cuprophan activated plasma (CAP) with (○) and without (●) continuous infusion of 0.05 mg/kg body wt/min of OKY1581.* Values are presented as mean ±SEM. *denotes differences with $P < 0.05$.

above. This small dose of OKY1581 was, however, unable to inhibit the pulmonary hypertension induced by intravenous bolus infusion of a larger dose (0.6 ml/kg body wt) of CAP (Fig. 2D); the pulmonary artery pressure increased by two-fold to 48 ± 6 mm Hg ($P < 0.01$). A representative tracing is shown in Figure 3.

To rule out the possibility that the abolishment of CAP-induced pulmonary hypertension seen after OKY1581 infusion was simply due to refractoriness of the animal response to repeat CAP challenge, experiments were conducted on a separate set of animals using normal saline as control instead of OKY1581. The data are presented in Table 1. Upon the initial 0.15 ml/kg CAP challenge, mean pulmonary artery pressure (PAP) increased from 27.0 ± 1.9 to 50.2 ± 2.8 mm Hg (N = 5, $P < 0.01$). After saline infusion for 30 minutes, the same CAP

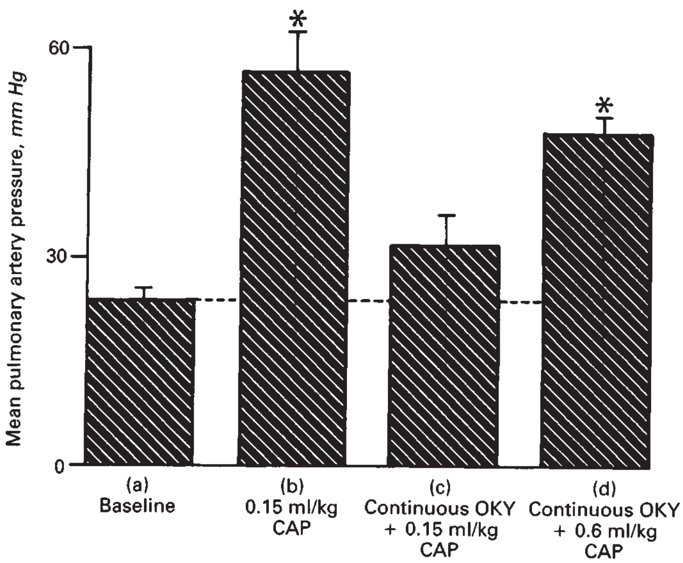

Fig. 2. *Mean pulmonary artery pressure (PAP) at baseline (A); 0.5 min after acute intravenous bolus infusion of 0.15 ml/kg body wt of CAP without (B); or with (C) continuous infusion of 0.05 mg/kg body wt/min of OKY1581; 0.5 min after 0.6 ml/kg body wt of CAP with 0.05 mg/kg body wt/min of continuous OKY1581 (D).* Values represent mean ±SEM in seven animals. * $P < 0.01$ vs. baseline.

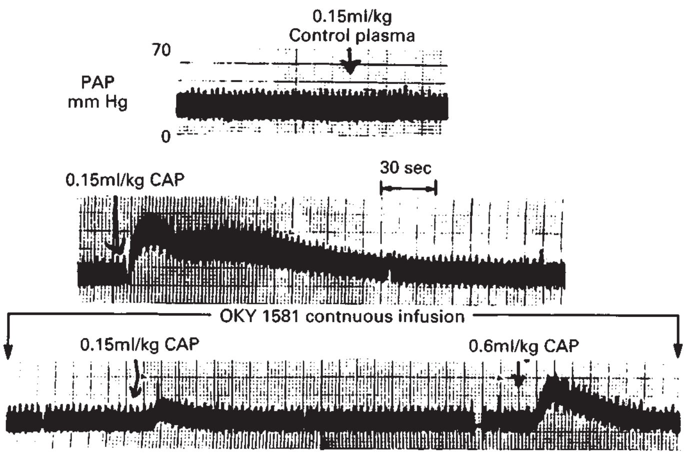

Fig. 3. *Representative tracings of the pulmonary artery pressure (PAP) in the swine in response to infusion of control plasma, 0.15 ml/kg body wt of CAP with or without continuous infusion of OKY1581, and 0.6 ml/kg body wt of CAP with OKY1581.*

challenge again increased the PAP from 27.2 ± 1.6 to 52.4 ± 4.0 mm Hg ($N = 5$, $P < 0.01$), demonstrating that there is no refractoriness of response to repeat CAP infusion after 30 minutes. In contrast, repeat challenge with CAP failed to elicit pulmonary hypertension after OKY1581 infusion as indicated in Figures 1, 2, 3 and Table 1.

Animal studies with ZAP

The effects of zymosan–activated plasma (ZAP) on pulmonary artery pressure and peripheral leukocyte counts are depicted in Figure 4. Intravenous bolus challenge with 0.2 ml/kg body wt of ZAP likewise induced an increase in mean pulmonary artery pressure (from 22.4 ± 1.4 to 56.1 ± 4.6 mm Hg, $P < 0.001$) and systemic arterial TXB_2 concentration (from 0.18 ± 0.08 to 0.66 ± 0.16 ng/ml, $P < 0.02$). However, in contrast to CAP, ZAP also induced a significant leukopenia (from 16.6 ± 2.9 × 10^3 cells/mm^3 to 8.1 ± 2.4 × 10^3 cells/mm^3, $P < 0.01$). Pretreatment with continuous infusion with OKY1581 abolished the pulmonary hypertension and the increase in thromboxane concentration, but not the leukopenia.

In vitro studies with porcine blood cells

To identify the potential sources of the thromboxane released by CAP infusion, porcine blood cells were incubated with CAP in vitro. Incubation of CAP with peripheral blood polymorphonuclear cells, mononuclear cells or platelets did not increase the plasma thromboxane concentration significantly (Table 2). Positive controls for polymorphs and mononuclear cells were provided by incubation of these cells with a mixture of arachidonic acid and calcium ionophore A23187. The thromboxane concentration in the media increased from 0.25 ± 0.08 to 1.92 ± 0.25 ng/ml ($N = 4; P < 0.01$) for polymorphs; the concentration increased from 0.53 ± 0.11 to 1.24 ± 0.16 ng/ml ($N = 6; P < 0.01$) for mononuclear cells. Positive controls for platelets were performed by incubation with collagen, which increased the thromboxane concentration from 0.18 ± 0.07 to 6.62 ± 1.53 ng/ml ($N = 4; P < 0.05$).

In vitro studies with other porcine tissues

When lung fragments were incubated with CAP in vitro for 30 minutes, the concentration of TXB_2 in the incubation medium increased from 0.25 ± 0.05 to 3.83 ± 0.45 ng/ml ($P < 0.01$) (Fig. 5). The addition of 10^{-3} M OKY1581 to the incubation medium abolished the increment (from 0.24 ± 0.33 to 0.21 ± 0.07 ng/ml, $P > 0.05$, $N = 4$). This suggests that in the absence of the synthetase inhibitor, the thromboxane was produced during the incubation with CAP, but not preformed and simply released by the lung tissues as a result of trauma.

Interestingly, incubation of lung fragments with platelet poor plasma in vitro for 30 minutes also resulted in an increment of plasma thromboxane concentration (0.33 ± 0.15 to 2.09 ± 0.17 ng/ml, $N = 4$; $P = 0.01$). This increment is, however, less than that observed with lung incubated with CAP.

Incubation of CAP with other porcine tissues yield variable results (Table 3). Incubation with left atrium, inferior vena cava, liver and spleen increased the plasma concentrations of thromboxane ($N = 4$; $P < 0.05$). These increments were, however, significantly lower than those induced by incubation of CAP with lung tissues ($N = 4$; $P < 0.02$).

Discussion

We and others have previously demonstrated that acute intravenous infusion of cuprophan–activated plasma (CAP) into experimental swine or sheep produced various cardiopulmonary changes including severe pulmonary hypertension [6–8]. Infusion of purified porcine $C5a_{desArg}$ into swine produced rather similar results [6], whereas heat inactivation of plasma prior to cuprophan exposure abolished the alteration in pulmonary physiology in sheep [7]. These two latter observations suggested that the CAP-induced pulmonary hypertension was mediated by complement activation products C3a, C5a and/or their des-Arginine derivatives.

Table 1. Changes in mean pulmonary artery pressure (PAP) induced by CAP infusion before and after infusion of OKY1581 or saline

Initial CAP challenge (N = 5)		Repeat CAP challenge after saline (N = 5)		Initial CAP challenge (N = 7)		Repeat CAP challenge after OKY1581 (N = 7)	
PAP (Pre)	PAP (Post)	PAP (Pre)	PAP (Post)	PAP (Pre)	PAP (Post)	PAP (Pre)	PAP (Post)
27.0 ±1.9	50.2[a] ±2.8	27.2 ±1.6	52.4[a] ±4.0	24.0 ±1.4	59.1[a] ±5.2	23.0 ±4.3	32.2 ±4.2

0.15 ml/kg CAP was given in each instance. Ten minutes after the initial CAP challenge, either saline or OKY1581 was given as continuous infusion for 30 minutes. The repeat CAP challenge was then given; Pre, before CAP challenge; Post, 30 seconds after CAP challenge.

[a] These values are not statistically different from each other; they are however significantly higher than their corresponding baseline values ($P < 0.01$).

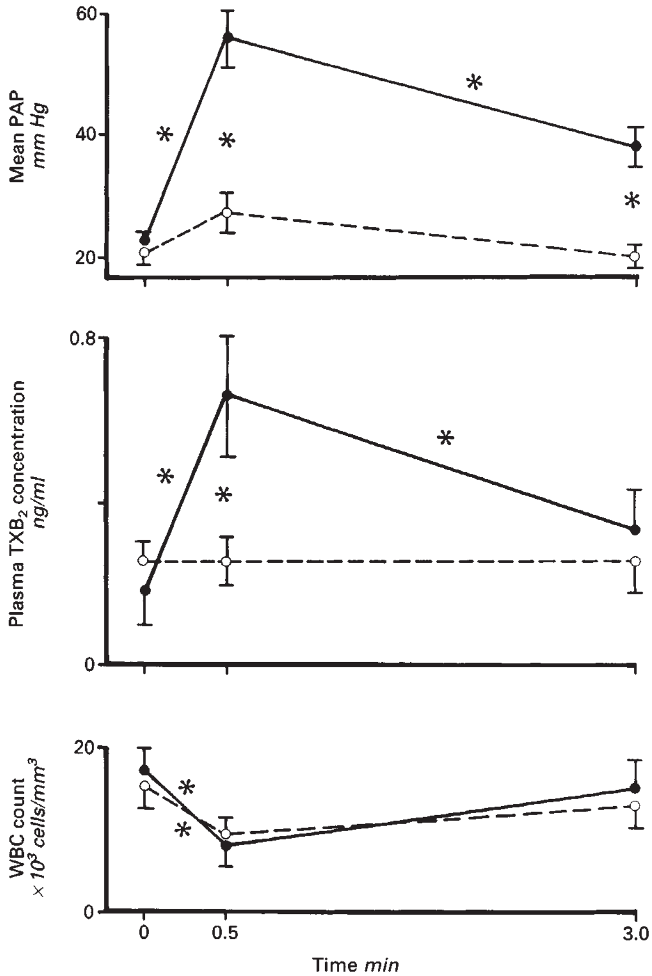

Fig. 4. *Responses of mean pulmonary artery pressure (PAP), systemic arterial plasma thromboxane $B_2(TXB_2)$ concentration, and peripheral leukocyte (WBC) counts to bolus infusion of 0.2 ml/kg body wt of zymosan activated plasma (ZAP) into experimental swine* (N = 7) *with* (○) *or without* (●) *continuous infusion of 0.05 mg/kg body wt/min of OKY1581.* Values are presented as mean ±SEM. *$P < 0.05$.

Table 2. Changes in plasma thromboxane concentrations following in vitro incubation of porcine blood cells with cuprophan-activated plasma (CAP) and controls

	CAP		AA ± Ca Ionophore		Collagen	
	0′	30′	0′	30′	0′	30′
	ng/ml		*ng/ml*		*ng/ml*	
PMN	0.24 ±0.03	0.36 ±0.10	0.25 ±0.08	1.92[a] ±0.25	—	—
MNC	0.34 ±0.05	0.37 ±0.05	0.53 ±0.11	1.24[a] ±0.16	—	—
PRP	0.29 ±0.06	0.29 ±0.04	—	—	0.18 ±0.07	6.62[b] ±1.53

Abbreviations are: PMN, peripheral polymorphonuclear cells; MNC, mononuclear cells; PRP, platelet rich plasma; AA arachidonic acid; Ca, calcium.

[a] $P < 0.01$ vs. baseline

[b] $P < 0.05$ vs. baseline

Indeed, it has been well established that complement is activated during clinical hemodialysis with cuprophan membranes with the resultant formation of anaphylatoxins C3a and C5a [10, 11]. However, radioimmunoassays for porcine or sheep anaphylatoxins are currently unavailable. Therefore, the production of anaphylatoxins in porcine or sheep blood by cuprophan exposure is only inferential.

The peptides C3a and C5a are termed anaphylatoxins because of their abilities to contract guinea-pig ileal smooth-muscles [12], increase vascular permeability of blood vessels [13] and cause histamine release from mast cells [14]. However, some of the biological actions of these peptides on end organs may be indirect. Human C3a had been shown to release thromboxane from guinea pig peritoneal-macrophages [15]. Both human and porcine C3a cause contraction of isolated guinea pig lung-strips, which can be blocked by indomethacin or aspirin [16]. Porcine $C5a_{desArg}$ has been reported to stimulate the release of leukotrienes from guinea pig lung fragments and to contract guinea pig lung strips. This latter effect could be inhibited by leukotriene inhibitor FPL55712 [17]. Thus the complement and arachidonic acid systems seem to be closely related to each other.

Recognizing some of these interactions between the complement activation products and arachidonic acid metabolites, and presuming that anaphylatoxins are formed during porcine plasma exposure to cuprophan, we investigated the roles of the cyclooxygenase metabolites of arachidonic acid in CAP-induced pulmonary hypertension in the present study. Indeed, there was a significant increment in plasma thromboxane concentration concomitant to the rise in pulmonary pressure in response to CAP infusion into the swine. The increase of both could be blocked by a thromboxane inhibitor OKY1581. On the

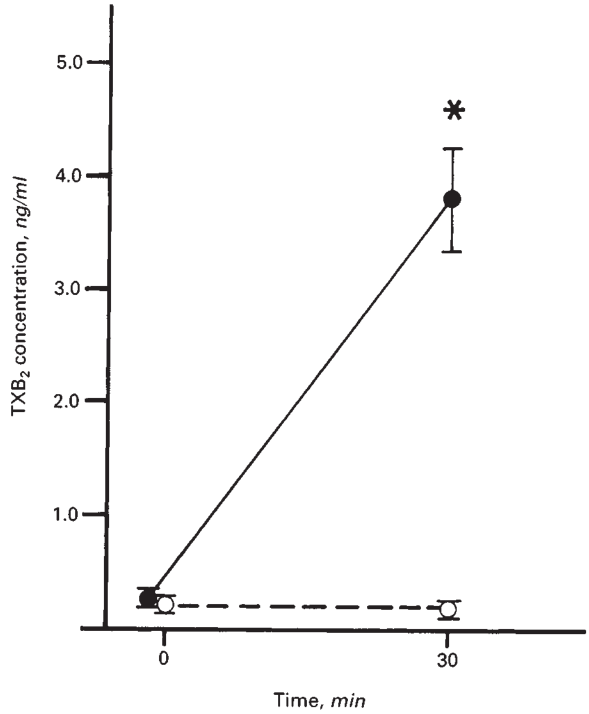

Fig. 5. *Changes in plasma concentration of thromboxane $B_2(TXB_2)$ when lung tissues were incubated with CAP in vitro.* Incubation was carried out with (○, $N = 5$) or without (●, $N = 5$) OKY1581. Values are represented as mean ±SEM. *$P < 0.01$ compared to baseline.

Table 3. Changes in plasma thromboxane concentrations following in vitro incubation of different porcine tissues with cuprophan–activated plasma (CAP)

	0' *ng/ml*	30' *ng/ml*	*P* *0' vs. 30'*	*P* *30' vs. lung 30'*
Lung	0.25 ± 0.05	3.83 ± 0.45	<0.01	—
Renal cortex	0.33 ± 0.06	0.38 ± 0.09	NS	<0.01
Renal medulla	0.26 ± 0.03	0.93 ± 0.26	NS	<0.01
Left atrium	0.25 ± 0.05	0.42 ± 0.06	<0.02	<0.01
Left ventricle	0.28 ± 0.03	0.28 ± 0.07	NS	<0.01
Pulmonary artery	0.31 ± 0.03	0.31 ± 0.06	NS	<0.01
Aorta	0.31 ± 0.08	0.30 ± 0.04	NS	<0.01
Inferior vena cava	0.33 ± 0.07	0.56 ± 0.07	<0.05	<0.01
Liver	0.40 ± 0.09	0.76 ± 0.06	<0.05	<0.01
Spleen	0.38 ± 0.06	1.67 ± 0.36	<0.05	<0.02

other hand, plasma PGE_2 did not change under the same conditions.

The compound OKY1581 had been demonstrated to be a potent and specific thromboxane synthetase–inhibitor [18]. It effectively blocks the production of thromboxanes associated with the aggregation of rabbit platelets, and simultaneously increased prostaglandin E and F generation. This is in contrast to the nonspecific inhibitory effects of indomethacin on the cyclooxygenase pathway. The present study strongly suggested that the thromboxanes were at least partly responsible for the CAP-induced pulmonary hypertension in the swine. This was in agreement with the study in which indomethacin blocked the pulmonary hypertension in the sheep induced by whole blood previously exposed to cuprophan membranes [9]. Plasma thromboxane concentrations were not reported in that study.

Despite the pulmonary hypertension, there was no statistically significant decrease in the peripheral leukocyte count in response to a small dose (0.15 ml/kg) of CAP infused in the present study. This was in accordance with the earlier observations of ours and others that the pulmonary leukosequestration did not cause the pulmonary hypertension [6, 9]. Instead, other vasoactive humoral factors such as anaphylatoxins and thromboxane were responsible. This absence of significant leukopenia also suggests that the amount of $C5a_{desArg}$ necessary to induce pulmonary vascular smooth muscle contraction is lower than that required for leukoagglutination. This is in accordance with the in vitro observations that the chemotactic capabilities of C5a and $C5a_{desArg}$ occur in the nanomolar range whereas smooth muscle contraction occurs even in subnanomolar concentrations [19, 20].

Zymosan is known to be a very potent alternative pathway activator of complement [21]. Infusion of zymosan activated plasma (ZAP) into the animals in the present study was also associated with increments in pulmonary artery pressure and plasma thromboxane concentrations, both of which could be blocked by the thromboxane synthetase inhibitor. This adds further support to our hypothesis that CAP-induced pulmonary hypertension is mediated by complement activation products and the thromboxanes. There was a minor difference between the results obtained by CAP and ZAP in the present study; the peripheral leukocyte counts dropped significantly with ZAP but not with CAP. This may be due to a difference in the amount of chemotactic anaphylatoxins contained in the injected plasma generated by exposure to two different types of surfaces. As noted above, we could not measure porcine anaphylatoxins directly. However, in our previous study employing recalcified human serum, the zymosan surface seemed to be a more potent activator of complement than the cuprophan surface [22].

It should be pointed out that other intermediates may also be involved in the pathogenesis of cuprophan-induced pulmonary hypertension. For example, porcine $C5a_{desArg}$ stimulates leukotriene release from guinea pig lung [17] as mentioned above and infusion of leukotriene C_4 increases the output of thromboxane A_2 from isolated guinea pig lung [23]. Thus it is conceivable that the leukotrienes are intermediates of the pulmonary hypertensive effects of complement fragments induced by plasma exposure to cuprophan.

We postulate that the abolishment of CAP-induced pulmonary hypertension by OKY1581 is due to thromboxane synthesis blockade. One may question if it is possible that OKY1581 shunts the arachidonic acid metabolism to the prostacyclin synthetase pathway so that the increased production of the vasodilatory prostacyclin is the mechanism by which OKY1581 blocks the pulmonary hypertension. This possibility is rather unlikely since the nonspecific cyclooxygenase inhibitor indomethacin also abolishes the pulmonary vasoconstrictive response [9].

Besides causing pulmonary hypertension, the thromboxane generated may also cause other cardiopulmonary alterations seen with CAP infusion in swine or sheep [6–8]. Intravenous injection of the thromboxane increased the tracheal insufflation pressure in guinea pigs [24] and increased airway pressure as well as pulmonary artery pressure in dogs [25]. Intraventricular administration of thromboxane into dogs also produced a mild elevation of systemic arterial pressure and mild depression of myocardial contractility [25]. These effects of thromboxanes are consistent with the pulmonary and systemic hypertension, hypoxemia as well as the rise in left ventricular end–diastolic pressure observed in swine in our earlier studies [6].

The sources of the thromboxane released by CAP in either the swine or sheep models are unknown. Since the pulmonary artery pressure rose within seconds after the CAP infusion, we postulated that the tissues releasing the thromboxanes must be within the blood or in close contact with the blood. In humans, platelets are rich in thromboxane [26], although other tissues such as polymorphonuclear cells [27] thoracic duct lymphocytes [28] and the lungs [28, 29] have been demonstrated to produce these metabolites. Sheep peripheral lymphocytes also produce thromboxane upon stimulus [30]. It should be noted that species differ in their abilities to release thromboxane by certain tissues [31]. We first examined the porcine peripheral blood cells by incubating them in vitro with CAP. None of the examined blood cell types, including polymorphonuclear and mononuclear cells as well as platelets, released thromboxane appreciably. They did, however, respond to the appropriate positive controls (arachidonic acids + calcium ionophore and collagen). Therefore, CAP did not seem to be an adequate stimulus to these hematogenous cells to produce thromboxane.

The solid tissues were next examined. We found that the lungs produced more thromboxane than any other tissues in response to CAP in vitro. The thromboxanes were synthesized by the lungs during and not before the incubation, since the addition of the thromboxane synthetase inhibitor at the beginning of the incubation period abolished the increments in plasma thromboxane concentrations. Trauma of the lung tissues during removal from the animals and slicing did not stimulate thromboxane production.

Interestingly, significant amounts of thromboxane were released by the lung tissues when incubated with porcine plasma not previously exposed to cuprophan, although the magnitude was significantly smaller than that produced by incubation with CAP. The reason for this release is unknown. Presumably the porcine lung tissues have a high tendency to synthesize thromboxane in response to different stimuli in vitro.

Incubation of CAP with other porcine solid tissues yielded variable results. Left atrium, inferior vena cava, liver and spleen seemed to generate thromboxane, but the magnitude was smaller than that generated by the lungs. The relative contributions of the thromboxane from these organs in mediating the CAP-induced pulmonary hypertension in intact animals are yet to be determined.

In summary, acute intravenous infusion of cuprophan activated plasma in the swine caused an increase in pulmonary artery pressure and plasma thromboxane concentration. These increases could be inhibited by a specific thromboxane synthetase inhibitor. In vitro studies suggest that this thromboxane was derived from various solid organs, with the lungs being the most abundant source. Based on the current and previous studies, we postulated the following sequence of events. Exposure of autologous porcine plasma to the cuprophan dialysis membranes results in formation of anaphylatoxins C3a, C5a and their desArg derivatives. When this cuprophan–activated plasma is infused into experimental swine, C5a or $C5a_{desArg}$ cause pulmonary leukosequestration and peripheral leukopenia. Complement C5a, C3a and/or their desArg derivatives also stimulate various organs to release thromboxane. These arachidonic acid metabolites in turn mediate the pulmonary hypertension and probably the hypoxemia seen in this model.

Acknowledgments

This study is supported by a Merit Review grant from the Veterans Administration and an Institutional grant from the National Institute of Health. The authors thank the personnel of the Animal Research Facility at the Veterans Administration Medical Center, Salt Lake City for technical assistance. This manuscript was presented in part at the American Society of Nephrology annual meeting, December 1984 and December 1985. The generous gift of OKY1581 from ONO Pharmaceutical Co., Ltd. and Kissei Co. is greatly appreciated.

Reprint requests to Alfred K. Cheung, M.D., Renal Section (11-H), VA Medical Center, 500 Foothill Blvd., Salt Lake City, Utah 84148, USA.

References

1. Kant GS, Pollak VE, Cathey M, Goetz D, Berlin R: Multiple use of dialyzers: Safety and efficacy. *Kidney Int* 19:728–738, 1981
2. Rosa AA, Fryd DS, Kjellstrand CM: Dialysis symptoms and stabilization in long–term dialysis: Practical application of the CUSUM plot. *Arch Int Med* 140:804–807, 1980
3. Quellhorst EA, Schuenemann B, Hildebrand U: Morbidity and mortality in long–term hemofiltration. *Asaio J* 6:185–191, 1983
4. Agar JW, Hull JD, Kaplan M, Pletka PG: Acute cardiopulmonary decompensation and complement activation during hemodialysis. *Ann Intern Med* 90:792–793, 1979
5. Walker JF, Lindsay RM, Driedger AA, Sibbald WJ, Linton AL: Hemodialysis commonly causes transient pulmonary hypertension. *(abstract) Am Soc Nephrol* 58A, 1983
6. Cheung AK, LeWinter M, Chenoweth DE, Henderson LW: Cardiopulmonary effects of cuprophan–activated plasma in the swine: Role of complement products. *Kidney Int* 29:799–806, 1986
7. Craddock PR, Fehr J. Brigham KL, Kronenberg RS, Jacob HS: Complement and leukocyte–mediated pulmonary dysfunction in hemodialysis. *N Engl J Med* 296:769–774, 1977
8. Walker JF, Lindsay RM, Peters SD, Sibbald WJ, Linton AL: A sheep model to examine the cardiopulmonary manifestations of blood–dialyzer interactions. *Asaio J* 6:123–130, 1983
9. Walker JF, Lindsay RM, Sibbald WJ, Linton AL: Acute pulmonary hypertension, leukopenia and hypoxia in early haemodialysis. *Proc EDTA-ERA* 21:135–142, 1984
10. Chenoweth DE, Cheung AK, Henderson LW: Anaphylatoxin formation during hemodialysis: Effects of different dialyzer membranes. *Kidney Int* 24:764–769, 1983
11. Chenoweth DE, Cheung AK, Ward DM, Henderson LW: Anaphylatoxin formation during hemodialysis: Comparison of new and re-used dialyzers. *Kidney Int* 24:770–774, 1983
12. Cochrane CG, Müller–Eberhard HJ: The derivation of two distinct anaphylatoxin activities from the third and fifth components of human complement. *J Exp Med* 127:371–386, 1968
13. Lepow IH, Willms-Kretschmer K, Patrick RA, Rosen FS: Gross and ultrastructural observations on lesions produced by intradermal injection of human C3a in man. *Am J Path* 61:13–24, 1970
14. Johnson AR, Hugli TE, Müller–Eberhard HJ: Release of histamine from mast cells by the complement peptides C3a and C5a. *Immunology* 28:1067–1080, 1975
15. Hartung H-P, Bitter–Suermann D, Hadding U: Induction of thromboxane release from macrophages by anaphylatoxic peptide C3a of complement and synthetic hexapeptide C3a 72-77. *J Immun* 130:1345–1349, 1983
16. Stimler NP, Bloor CM, Hugli TE: C3a-induced contraction of guinea pig lung parenchyma: Role of cyclooxygenase metabolites. *Immunopharmacology* 5:251–257, 1983

17. Stimler NP, Back MK, Bloor CM, Hugli TE: Release of leukotrienes from guinea pig lung stimulated by $C5a_{desArg}$ anaphylatoxin. *J Immunol* 128:2247–2252, 1982
18. Smith JB, Jubiz W: OKY-1581: A selective inhibitor of thromboxane synthesis in vivo and in vitro. *Prostaglandins* 22:353–363, 1981
19. Gerard C, Hugli TE: Anaphylatoxin from the fifth component of porcine complement: Purification and partial chemical characterization. *J Biol Chem* 254:6346–6351, 1979
20. Gerard C, Hugli TE: Identification of classical anaphylatoxin as the des-Arg form of the C5a molecule: Evidence of a modulator role for the oligosaccharide unit in human des-Arg^{74}-C5a. *Proc Natl Acad Sci USA* 78:1833–1837, 1981
21. Müller-Eberhard HJ, Schreiber RD: Molecular biology and chemistry of the alternative pathway of complement. *Adv Immunol* 29:1–53, 1980
22. Cheung AK, Chenoweth DE, Otsuka D, Henderson LW: Compartmental distribution of complement activation products in artificial kidneys. *Kidney Int* 30:74–80, 1986
23. Folco G, Hansson G, Graström E: Leukotriene C_4 stimulates TXA_2 formation in isolated sensitized guinea pig lungs. *Biochem Pharm* 30:2491–2493, 1981
24. Svensson J, Strandberg K, Tuvemo T, Hamberg M: Thromboxane A_2: Effects on airway and vascular smooth muscle. *Prostaglandins* 14:425–436, 1977
25. Friedman LS, Fitzpatrick TM, Bloom MF, Ramwell PW, Rose JC, Kot PA: Cardiovascular and pulmonary effects of thromboxane B_2 in the dog. *Circ Res* 44:748–751, 1979
26. Needleman P, Minkes M, Raz A: Thromboxanes: Selective biosynthesis and distinct biological properties. *Science* 193: 163–165, 1976
27. Goldstein IM, Malmsten CL, Kaplan HB, Kindahl H, Samuelsson B, Weissman G: Thromboxane generation by stimulated human granulocytes: Inhibition by glucocorticoids and superoxide dismutase. (abstract) *Clin Res* 25:518A, 1977
28. Morley J, Bray MA, Jones RW, Nugteren DH, van Dorp DA: Prostaglandin and thromboxane production by human and guinea–pig macrophages and leucocytes. *Prostaglandins* 17: 729–736, 1979
29. Hopkins NK, Sun FF, Gorman RR: Thromboxane A_2 biosynthesis in human lung fibroblasts WI-38. *Biochem Biophys Res Commun* 85, No 2:827–836, 1978
30. Tahamont MV, Gee MH, Flynn JT: Aggregation and thromboxane synthesis and release in isolated sheep neutrophils and lymphocytes in response to complement stimulation. *Prostaglandins Leukotrienes Med* 16:181–190, 1984
31. Leach CM, Thorburn GD: A comparative study of collagen induced thromboxane release from platelets of different species: Implications for human atherosclerosis models. *Prostaglandins* 24:47–59, 1982